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Determination of the fatty acid profile by ^{1}H-NMR spectroscopy*

The common unsaturated fatty acids present in many vegetable oils (oleic, linoleic and linolenic acids) can be quantitated by ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR). A key feature is that the signals of the terminal methyl group of linolenic acid are shifted downfield from the corresponding signals in the other fatty acids, permitting their separate integration and quantitation of linolenic acid. Then, using the integration values of the signals of the allylic and *bis*-allylic protons, oleic and linoleic acids can be quantitated. The procedure was verified for mixtures of triacylglycerols (vegetable oils) and methyl esters of oleic, linoleic and linolenic acids as well as palmitic and stearic acids. Generally, the NMR (400 MHz) results were in good agreement with gas chromatographic (GC) analyses. As the present ^{1}H-NMR-based procedure can be applied to neat vegetable oils, the preparation of derivatives for GC would be unnecessary. The present method is extended to quantitating saturated (palmitic and stearic) acids, although in this case the results deviate more strongly from actual values and GC analyses. Alternatives to the iodine value (allylic position equivalents and *bis*-allylic position equivalents) can be derived directly from the integration values of the allylic and *bis*-allylic protons.

Keywords: Fatty acid methyl esters, fatty acid profile, gas chromatography, nuclear magnetic resonance, triacylglycerols, vegetable oils.

1 Introduction

The fatty acid profile of vegetable oils, animal fats and their derivatives such as alkyl esters is the major factor influencing their chemical and physical properties. Consequently, the fatty acid profile strongly influences the application of fats and oils for physiological as well as industrial uses such as lubricants and biodiesel. Therefore, numerous methods have been developed to determine this profile.

Gas chromatography (GC) is among the most common methods for determining the fatty acid composition of vegetable oils, animal fats and their derivatives. For that purpose, the oils or fats are usually converted to the corresponding methyl esters. Spectroscopic methods, on the other hand, yield information on all components of a mixture in one spectrum, usually without the need to derivatize or destroy the sample. Spectroscopic methods may also be useful for samples which are not amenable to chromatographic methods due to, for example, heat sensitivity or other factors. As a result of factors such as overlapping peaks, quantitating individual components in mixtures by spectroscopic methods may be difficult. The present paper outlines a method by which information on the amounts of individual fatty acids can be obtained by means of ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectroscopy in an oil or fat sample or in form of derivatives such as methyl esters.

Probably the first report on the ^{1}H-NMR spectra of fatty compounds was published in 1959 [1]. Three years later, the presumably first report using ^{1}H-NMR for quantitatively determining unsaturation and average molecular weight followed [2]. Besides related reports on determining the iodine value [3], the use of ^{1}H-NMR has since expanded to include the identification of vegetable oils as well as identifying individual vegetable oils in mixtures thereof [4–6] and the determination of specific fatty acids in materials such as fish oils [7, 8]. ^{13}C-NMR has been used for similar purposes as ^{1}H-NMR in the analysis of fatty compounds [9–16]. Both ^{1}H [17–19] and ^{13}C-NMR [17] have been applied to the determination of fatty acid composition. Several papers containing reviews of these aspects were published in recent years [20–29].

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Three papers report on the use of ^{1}H-NMR for determining the amounts of specific fatty acids in vegetable oils [17–19]. While one investigation [19] used a relaxation technique to determine oleic (C18:1) and linoleic (C18:2) acid in sunflower oil, another study [17] employed the integration values of relevant peaks, among them those of the glyceridic protons and very recently unsaturated fatty acids in prepared triacylglycerol mixtures were determined from the integration values of protons on unsaturated carbons [18]. Besides these investigations, assignments of the salient peaks to specific protons can be found in the literature [30]. In the present paper, a method for determining fatty acid composition utilizing only the integration values peaks caused by the protons in the fatty acid chains is reported. The present method uses equations not applying the integration area per proton derived from the full spectrum as employed previously [17]. It is therefore not only suitable for triacylglycerols [18] as found in neat oils and fats but also for derivatives such as methyl esters. The signals of all protons are well-separated, thus permitting their straightforward integration and use in calculating fatty acid composition. The method was verified for samples containing mixtures of methyl esters and triacylglycerols. The method can theoretically be extended to determining the saturated fatty acids palmitic (C16:0) and stearic (C18:0) acids using the integration value of the methylene protons in the fatty acid chains, however, this procedure is very sensitive to small variations in the integration values and gives results that can vary considerably from the known composition. A method for calculating alternatives to the iodine value, allylic and *bis*-allylic position equivalents [31], from ^{1}H-NMR data is also discussed.

2 Materials and methods

Methyl soyate was obtained from *Ag Environmental Products* (trade name *SoyGold*, Lenexa, KS, USA). Pure (>99% as verified by GC-MS analyses of random samples) fatty acid methyl esters and triacylglycerols were purchased from *NuChek Prep*, Inc. (Elysian, MN, USA) and were used in the preparation of mixtures. Samples of refined vegetable oils were a gift from *Kathleen Warner* (USDA/ARS/NCAUR). Vegetable oils were transformed to methyl esters prior to GC analysis by a standard procedure using methanolic HCl. Samples referred to as "prepared" were obtained by accurate weighing on a balance and the prepared ratios are given.

400 MHz ^{1}H-NMR spectra were obtained under ambient conditions on a *Bruker* (Billerica, MA, USA) ARX-400 spectrometer using $CDCl_3$ as solvent which also served as internal reference (shift value of residual proton at 7.27 ppm).

2.1 Gas chromatography of fatty acid esters

Analysis of fatty acid esters was performed with a *Hewlett-Packard* 5890 Series II gas chromatograph (Palo Alto, CA, USA), equipped with a flame-ionization detector and a *Supelco* (Bellefonte, PA, USA) SP-2560 capillary column, (100 m × 0.25 mm i.d., 0.2 μm film thickness). The oven temperature ramp program was 175 °C for 5 min, 175–250 °C at 4 °C/min, and held for 20 min at 250 °C. Retention times were verified against authentic samples of individual pure fatty acid methyl esters. All relative percentages determined by GC for each fatty acid methyl ester sample are the means of triplicate runs. This method was used for the methyl esters reported in Tab. 1 and is termed method GC-1 in Tab. 2.

To compare the accuracy of GC *vs.* NMR, the methyl esters derived from vegetable oils (method GC-2 in Tab. 2) samples were additionally analyzed by a second gas chromatographic method utilizing a *Varian* (Palo Alto, CA, USA) 3400 CX gas chromatograph equipped with a flame-ionization detector and a *Supelco* SP-2380 capillary column (30 m × 0.25 mm; 0.2 μm film thickness). The oven temperature ramp program was 150 °C for 15 min, ramp 150–210 °C at 2 °C/min, 50 °C/min-220 °C with final 5-min hold time.

3 Results and discussion

The ^{1}H-NMR spectrum of mixtures of fatty compounds such as triacylglycerols as found in vegetable oils as well as animal fats and methyl esters are characterized by several salient regions of the fatty acid chains containing the signals of specific types of protons (see Fig. 1 for a spectrum of methyl soyate). The signals of these types of protons in the ^{1}H-NMR spectra of vegetable oils were used to quantitate individual unsaturated fatty acids [17]. The glyceridic protons, however, entered into the calculation [17]. In this paper, a method for quantitating fatty acids in a mixture is presented which does not use the protons of either the glycerol or methyl ester moieties and which is therefore applicable to neat oils and fats as well as derivatives such as methyl esters. The method is applied to mixtures containing the triacylglycerol or alkyl esters of palmitic (hexadecanoic; C16:0), stearic (octadecanoic, C18:0), oleic (9(*Z*)-octadecenoic; C18:1), linoleic (9(*Z*),12(*Z*)-octadecadienoic; C18:2) and linolenic (9(*Z*),12(*Z*),15(*Z*)-octadecatrienoic; C18:3) acids. The present method can theoretically be applied to oils or fats containing other fatty acids

Tab. 1. Fatty acid profiles of methyl soyate and prepared mixtures of fatty acid methyl esters by ^{1}H-NMR and GC (mean values and standard deviations (in parentheses) of triplicate determinations).

C16:0 + C18:0		C18:1		C18:2		C18:3	
Prepared	NMR (total saturates) GC	Prepared	NMR GC	Prepared	NMR GC	Prepared	NMR GC
Methyl soyate	16.55 (0.13) 11.00 (0.26) + 4.23 (0.13)	–	22.80 (0.85) 21.49 (0.11)	–	51.96 (1.47) 55.18 (0.37)	–	8.68 (0.69) 8.03 (0.05)
10.10+4.98	15.84 (0.32) 9.76 (0.06) + 4.89 (0.15)	20.04	19.80 (0.17) 19.53 (0.10)	54.91	54.77 (0.65) 55.95 (0.32)	9.97	9.58 (0.56) 9.88 (0.03)
10.03+4.99	15.86 (0.32) 9.69 (0.03) + 4.89 (0.03)	25.07	25.57 (0.71) 25.19 (0.07)	59.91	58.58 (0.51) 60.24 (0.11)	0	0 (–) 0 (–)
15.04+9.96	25.89 (0.67) 14.70 (0.09) + 9.85 (0.02)	19.91	20.14 (0.40) 20.08 (0.05)	55.09	53.97 (0.68) 55.37 (0.03)	0	0 (–) 0 (–)
9.97+4.94	16.36 (0.09) 9.81 (0.07) + 4.95 (0.03)	49.54	47.69 (0.27) 49.70 (0.02)	0	2.71 (0.41) 0.33 (0.01)	35.55	33.25 (0.12) 35.20 (0.04)
10.05+4.99	16.31 (0.17) 9.94 (0.05) + 5.01 (0.01)	59.96	58.71 (0.39) 60.07 (0.03)	0	1.28 (0.56) 0.29 (0.02)	25.01	23.69 (0.12) 24.70 (0.02)
45.03+24.98	71.27 (0.16) 44.97 (0.40) + 24.90 (0.40)	19.99	19.21 (0.23) 20.37 (0.18)	0	0.38 (0.56) 0 (–)	10.00	9.13 (0.21) 9.76 (0.10)
15.00+9.98	26.15 (0.44) 14.78 (0.04) + 9.95 (0.04)	30.06	29.68 (0.34) 30.18 (0.05)	39.99	39.57 (0.08) 40.23 (0.01)	4.98	4.60 (0.20) 4.86 (0.00)
21.03+5.25	28.11 (0.60) 20.68 (0.07) + 5.21 (0.02)	42.05	40.98 (0.46) 42.20 (0.04)	21.18	21.07 (0.74) 21.55 (0.05)	10.50	9.83 (0.32) 10.36 (0.05)
10.01+4.99	16.20 (0.51) 9.84 (0.03) + 4.92 (0.01)	0	0.20 (0.21) 0.13 (0.11)	64.91	64.53 (0.42) 65.16 (0.05)	20.09	19.08 (0.28) 19.95 (0.06)
10.03+4.99	16.24 (0.51) 9.85 (0.02) + 4.98 (0.02)	0	0.15 (0.42) 0.21 (0.01)	69.93	69.24 (0.90) 70.00 (0.03)	15.05	14.37 (0.32) 14.95 (0.01)
19.99+14.96	36.32 (0.11) 19.63 (0.18) + 15.05 (0.09)	0	0.62 (0.50) 0 (–)	54.99	53.35 (0.50) 55.34 (0.09)	10.06	9.71 (0.14) 9.98 (0.01)
0+0	0.25 (0.18) 0 (–) + 0 (–)	25.02	25.18 (0.39) 25.08 (0.01)	59.86	60.06 (0.81) 60.02 (0.03)	15.13	14.51 (0.35) 14.90 (0.03)
0+0	0.11 (0.32) 0 (–) + 0 (–)	29.97	30.22 (0.18) 29.99 (0.01)	60.02	59.85 (0.56) 60.19 (0.01)	10.01	9.82 (0.17) 9.81 (0.02)
0+0	0.47 (0.29) 0 (–) + 0 (–)	24.97	24.81 (0.60) 25.04 (0.04)	65.02	65.26 (0.83) 65.15 (0.06)	10.00	9.46 (0.18) 9.81 (0.02)

Tab. 2. Fatty acid composition by two GC methods and ^{1}H-NMR of vegetable oils and their methyl esters (ME) as well as ^{1}H-NMR analysis of some prepared triacylglycerol mixtures. The methyl esters were derived directly from the oils for purpose of GC analysis.

Sample	Method	C14:0	C16:0	C18:0	C18:1	C18:2	C 18:3
Vegetable oil samples							
Cottonseed oil ME	GC-1	0.73	24.69	2.60	17.77	54.14	0.07
	GC-2	0.55	26.06	2.48	16.11+0.8+0.12[‡]	53.10+0.54[#]	0.24
Cottonseed oil	NMR[†]	–	–	–	19.24	49.97	0.00
High-oleic safflower oil ME	GC-1	0.00	4.87	2.10	79.18	13.64	0.20
	GC-2	0.05	5.21	1.98	79.39+0.35[‡]	12.78	0.25
High-oleic safflower oil	NMR[†]	–	–	–	79.22	12.13	0.00
Soybean oil ME	GC-1	0.00	10.73	4.66	24.01	53.15	7.44
	GC-2	0.07	11.83	4.46	22.53+1.33[§]	52.42	7.35
Soybean oil	NMR[†]	–	–	–	24.5	49.99	7.88
Mid-oleic sunflower oil ME	GC-1	0.00	4.89	3.68	57.55	33.44	0.44
Mid-oleic sunflower oil	GC-2	0.02	5.21	1.98	57.50+0.43[§]	32.75+0.18[#]	0.23
	NMR[†]	–	–	–	58.25	31.65	0.00
Prepared triacylglycerol samples			C16:0 + C18:0		C18:1	C18:2	C18:3
Prepared			14.97		19.99	54.91	10.13
	NMR		17.73		21.31	51.25	9.71
Prepared			15.05		25.08	59.88	0
	NMR		16.56		25.36	58.08	0
Prepared			14.94		50.09	0	34.97
	NMR		16.67		50.46	−0.08	32.95
Prepared			0		25.00	64.87	10.13
	NMR		1.36		26.15	62.31	10.19

[†] Total saturates by NMR: Cottonseed oil: 30.79%, high-oleic safflower oil: 8.65%, soybean oil: 17.63%, mid-oleic sunflower oil: 10.10%.
[‡] C18:1Δ9 + C18:1Δ11 + C18:1Δ13.
[#] C18:2-all-*cis* + C18:2-all-*trans*.
[§] C18:1Δ9 + C18:1Δ11.

as well, however, it can be complicated by the presence of several fatty acid chains of the same unsaturation type, for example, in (high-erucic) rapeseed oil which contains C18:1 and C20:1 fatty acid chains besides C22:1. Similarly, for oils containing significant amounts of more than two saturated fatty acid chains, for example lauric oils such as coconut and palmkernel oils, the present approach cannot give definitive results for the saturated fatty acid chains as it only provides results when there are two major saturated fatty acid chains present, however, the unsaturated fatty acids can still be quantitated. Besides chain length, other structural features possibly influencing the determination of the fatty acid profile by ^{1}H NMR as discussed here are the presence of fatty acids of same chain length but differing double bond positions or double bond configurations (*cis vs. trans*), branching of the chain as it can occur in animal fats, or changes in the fatty acid profile due to partial hydrogenation. A detailed investigation of these factors is beyond the scope of the present work. A procedure applied to prepared mixtures of triacylglycerols related to the one presented here was published recently [18].

The possible peaks in ^{1}H-NMR for quantitating unsaturated fatty acids are those of the olefinic protons (5.3–5.4 ppm), protons attached to the *bis*-allylic carbons (2.7–2.8 ppm), protons attached to the allylic carbons (2.0–2.1 ppm) and the terminal methyl group protons (0.8–0.9 ppm). Furthermore, the amounts of saturated fatty acids can be determined by utilizing the signal of the methylene (CH_2) protons at 1.2–1.4 ppm, although in this case, greater deviations were observed for the indi-

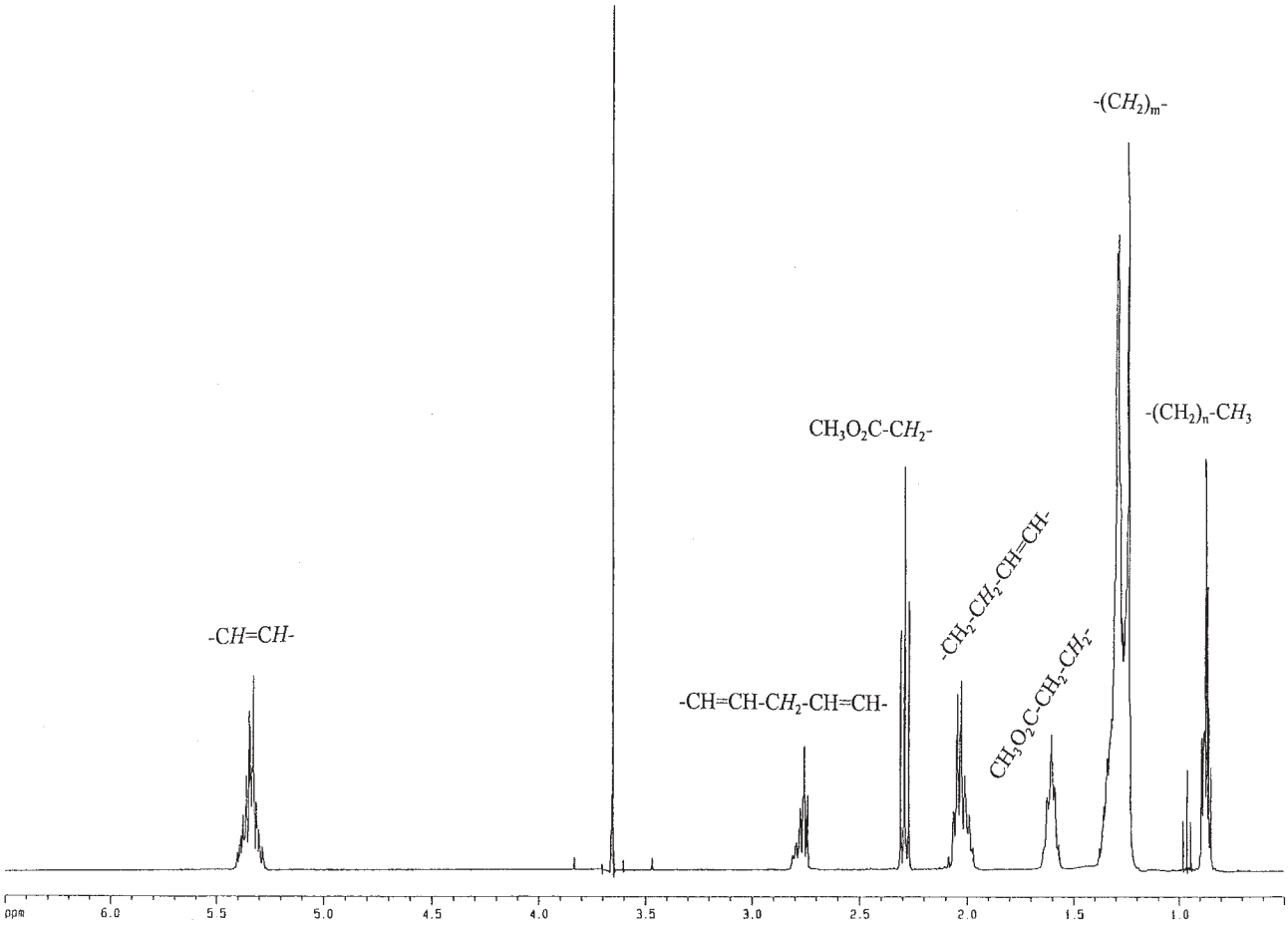

Fig. 1. ^{1}H-NMR spectrum of methyl soyate. With the exception of the peak of the methyl ester protons, the assignments to the individual signals are inscribed.

vidual saturated fatty acids (C16:0, C18:0) studied. However, the procedure satisfactorily reflects the overall amount of saturated fatty acids in a sample. The present equations hold for individual fatty acid chains, *i.e.*, they are directly applicable to fatty acid methyl esters. However, for triacylglycerols (vegetable oils), the integration values may need to be adjusted depending on which peak is chosen as reference and/or the value to which the reference signal is set due to the observation that the number of protons in the fatty acid chains per molecule triacylglycerol is 3 times that of methyl esters.

3.1 Quantitation of unsaturated fatty acids

The maximum theoretical integration values (= number of protons) for the olefinic (one proton per olefinic carbon), allylic, and *bis*-allylic protons (two protons per allylic or *bis*-allylic carbon) of C18:1, C18:2 and C18:3 fatty acids are given in Tab. 3.

Tab. 3. Maximum integration values for the specific protons of unsaturated fatty acids. One proton is given an integration value = 1.

Protons	oleic acid (C18:1)	linoleic acid (C18:2)	linolenic acid (C18:3)
olefinic	2	4	6
allylic	4	4	4
bis-allylic	0	2	4

The experimentally observed integration values for these peak regions are given by:

$$I_{exper,olefinic} = (A_{C18:1} \times I_{C18:1,olefinic}) + + (A_{C18:2} \times I_{C18:2,olefinic}) + (A_{C18:3} \times I_{C18:3,olefinic}) \quad (1)$$

$$I_{exper,allylic} = (A_{C18:1} \times I_{C18:1,allylic}) + (A_{C18:2} \times I_{C18:2,allylic}) + + (A_{C18:3} \times I_{C18:3,allylic}) \quad (2)$$

$$I_{exper,bisallylic} = (A_{C18:2} \times I_{C18:2,bisallylic}) + \\ + (A_{C18:3} \times I_{C18:3,bisallylic}) \quad (3)$$

in which I_{exper} corresponds to the experimentally determined integration value of the peak region given by the second term in the subscript, A corresponds to the amount of the mono-, di- and triunsaturated fatty acids (indicated by the C18:1, C18:2, and C18:3 subscripts) in the sample on a basis of 100% concentration of a neat component = 1(decimal values), and $I_{18:1}$, $I_{18:2}$, and $I_{18:3}$ are the maximum theoretical integration values given in Tab. 3. Eq. 3 contains only two terms since monounsaturated fatty acids lack *bis*-allylic carbons (indicated by 0 in Tab. 3). Using the values given in Tab. 3, Eqs. 1–3 become:

$$I_{exper,olefinic} = 2A_{C18:1} + 4A_{C18:2} + 6A_{C18:3} \quad (4)$$

$$I_{exper,allylic} = 4A_{C18:1} + 4A_{C18:2} + 4A_{C18:3} \quad (5)$$

$$I_{exper,bisallylic} = 2A_{C18:2} + 4A_{C18:3} \quad (6)$$

Eqs. 4–6 constitute 3 equations with 3 unknowns, as the integration values are determined experimentally. However, the signal of the terminal methyl groups can be used for determining the amount of linolenic acid (C18:3) instead of Eqs. 4–6. With the proximity of the C15-C16 double bond in linolenic acid to the terminal CH_3, the signal of the terminal CH_3 is shifted downfield to approximately 0.95 ppm (see Fig. 1) and can be integrated separately from the signal of terminal CH_3 in the other fatty acid chains. Thus, $A_{C18:3}$ is given by:

$$A_{C18:3} = I_{exper,methyl,C18:3}/(I_{exper,methyl,C18:3} + I_{exper,methyl,rest}) \quad (7)$$

in which $I_{exper,methyl,C18:3}$ is the integration value of the terminal CH_3 protons of linolenic acid and $I_{exper,methyl,rest}$ is the integration value of the terminal CH_3 protons of all other fatty acids in the sample.

With $A_{C18:3}$ determined in this fashion, $A_{C18:2}$ can be easily calculated by solving Eq. 6 for $A_{C18:2}$

$$A_{C18:2} = 0.5\ (I_{exper,bisallylic} - 4A_{C18:3}) \quad (8)$$

$A_{C18:1}$ can then be determined from either Eq. 4 or Eq. 5 rewritten as Eqs. 9 and 10, respectively, with Eq. 10 being more convenient. Thus:

$$A_{C18:1} = (I_{exper,olefinic} - 4A_{C18:2} - 6A_{C18:3})/2 \quad (9)$$

or

$$A_{C18:1} = (I_{exper,allylic}/4) - A_{C18:2} - A_{C18:3} \quad (10)$$

The total amount of unsaturated fatty compounds A_{unsat} in a sample using results from Eqs. 7, 8 and 10 is:

$$A_{unsat} = A_{C18:1} + A_{C18:2} + A_{C18:3} \quad (11)$$

The total amount of unsaturated fatty compounds can also be easily determined from the signal of the allylic protons giving the same result as Eq. 11 which arises from solving Eq. 5 for $I_{exp,allylic}$ and substituting Eq. 11 therein:

$$A_{unsat} = I_{exper,allylic}/4 \quad (12)$$

The allylic protons are more convenient for this determination because the number of allylic protons in C18:1, C18:2 and C18:3 is identical (Tab. 3) while the number of olefinic and *bis*-allylic protons differs.

The integration values of the olefinic, allylic, and *bis*-allylic protons are connected by the equation:

$$I_{exper,allylic} = 2I_{exper,olefinic} - 2I_{exper,bisallylic} \quad (13)$$

This result is obtained by solving Eq. 6 for $A_{C18:2}$, inserting the result in Eqs. 4 and 5 and solving for $I_{exper,olefinic}$ and $I_{exper,allylic}$, respectively. Solving the equation thus obtained for $I_{exper,olefinic}$ instead for $A_{C18:1}$, multiplying by 2 and inserting this result into the equation for $I_{exper,allylic}$ also derived as described in the previous sentence yields Eq. 13.

3.2 Quantitation of saturated fatty acids

The total amount of saturates in a sample is:

$$A_{sat} = 100 - A_{unsat} \quad (14)$$

^{1}H-NMR can theoretically also be applied to quantitating the saturates in a sample when assuming that there are only two major saturates present, for example, C16:0 and C18:0 as assumed here. For the quantitation of the saturates, only the integration values of the large methylene (CH_2) proton signal at 1.2–1.4 ppm are suitable because only in this case the varying amounts of CH_2 protons resulting from the different chain lengths of saturated compounds enter into an equation. The signals of other protons such as the protons α to the carboxyl function or the terminal methyl protons are not suitable since they are caused by the same number of protons from each fatty acid regardless of chain length.

The smaller CH_2 proton signals at approximately 1.6 ppm and 2.3 ppm are caused by two CH_2 protons each, namely those β to the carboxyl group (C-3) and α to the carboxyl group (C-2), respectively. They are well-separated from the signal of the other CH_2 protons and therefore do not need to be taken into consideration for quantitation. Also, taking the integration values of an increasing number of protons into account increases the potential for experimental error. Indeed, taking the C-3 protons into account reduced the accuracy of the evaluation of the saturated components as determined for a few samples (results not reported).

The following procedure is based on considering the proportional contribution of the CH_2 protons in the various fatty acid chains to the remaining large CH_2 signal at 1.2–1.4 ppm. Since the C-2 and C-3 protons do not contribute to the large CH_2 proton signal, the number of protons contributing to it are as follows: 24 for C16:0, 28 for C18:0, 20 for C18:1, 14 for C18:2 and 8 for C18:3. These numbers vary with fatty acid chain length, for example, for C22:1 a total of 28 protons contributes to the methylene signal at 1.2–1.4 ppm.

With unsaturates quantitated, it is necessary to multiply the amounts of the individual unsaturates with the number of contributing CH_2 protons to determine the (theoretical) integration value of the CH_2 protons of the unsaturates. Then this integration value is subtracted from the experimental CH_2 integration value to give the contribution of the CH_2 protons of the saturates. Thus:

$$I_{CH_2,C18:1} = 20 \times A_{C18:1} \quad (15)$$

$$I_{CH_2,C18:2} = 14 \times A_{C18:2} \quad (16)$$

$$I_{CH_2,C18:3} = 8 \times A_{C18:3} \quad (17)$$

$$I_{CH_2,C18:1} + I_{CH_2,\,C18:2} + I_{CH_2,\,C18:3} \quad (18)$$

$$I_{CH_2,sat} = I_{exper,CH_2,total} - I_{CH_2,unsat} \quad (19)$$

Theoretically, the remaining part of the CH_2 integration value can be contributed completely by either C16:0 or C18:0. The corresponding equations are:

$$I_{theor,CH_2,C16:0} = 24 \times A_{sat} \quad (20)$$

$$I_{theor,CH_2,C18:0} = 28 \times A_{sat} \quad (21)$$

The difference between the theoretical (for pure C16:0 and pure C18:0) and actual integration values is determined by:

$$I_{diff,\,CH_2,\,C16:0} = I_{CH_2,\,sat} - I_{theor,\,CH_2,\,C16:0} \quad (22)$$

$$I_{diff,\,CH_2,\,C18:0} = I_{theor,\,CH_2,\,C18:0} - I_{C_2,sat} \quad (23)$$

The difference between the theoretical integration values for contribution by either only C16:0 or C18:0 is obtained by subtracting Eq. 22 from Eq. 23:

$$I_{C18:0–C16:0} = I_{diff,\,CH_2,C18:0} - I_{diff,\,CH_2,C16:0} \quad (24)$$

The amount of C16:0 is then derived by determining its actual contribution to the integration value of the CH_2 proton signal from Eqs. 22 and 24 and multiplying this by the amount of saturates A_{sat} *from Eq. 14.*

$$A_{C16:0} = (I_{diff,CH_2,C16:0/C18:0–16:0}) \times A_{unsat} \quad (25)$$

Multiplication of the result from Eq. 25 with the factor 100 gives the percentage of C16:0. The amount of C18:0 is then:

$$A_{C18:0} = A_{sat} - A_{C16:0} \quad (26)$$

3.3 Experimental results

Tab. 1 contains results from the triplicate determination of fatty acid composition of methyl esters by ^{1}H-NMR and GC. These samples include methyl soyate and prepared mixtures of fatty acid methyl esters of known composition. The standard deviations included in Tab. 1 show that the repeatability of the NMR determination is usually reduced compared to GC. Tab. 2 contains results from the determination of the composition of samples containing triacylglycerols (vegetable oils and samples prepared from neat triacylglycerols such as triolein). For comparison of two different GC methods *vs.* ^{1}H-NMR with GC, the methyl esters of the vegetable oil samples in Tab. 2 were analyzed by a second GC method on another GC instrument. The results show that the reproducibility of GC analysis between instruments differs in some cases almost as much as the comparison of GC with the present ^{1}H-NMR method.

Generally, the results of ^{1}H-NMR and gas chromatographic determination of the unsaturated fatty acids are in good agreement. GC results are reported in area-% equated with wt-% while NMR data are reported in mol-%. The differences, however, are minor. It was reported that in the determination of fatty acid composition of vegetable oils, results from ^{13}C evaluation agreed better with GC results than those from ^{1}H-NMR [17]. It must be noted, however, that the results for quantitation of C18:1 and C18:2 can vary slightly depending on which peaks are used as reference in integration. The amounts of C18:3 will not vary as the integration ratio of the signals of terminal methyl of C18:3 *vs.* the signals of non-C18:3 does not change. Consistently using the same peak(s) as reference is therefore advisable. Another factor introducing deviations is that many vegetable oils contain small amounts of other unsaturated fatty acids besides the common ones assumed here. On the other hand, if it is known or assumed that a sample contains up to 3 unsaturated fatty acid derivatives other than those discussed here, the method can be appropriately modified by changing the number of protons entering into the equations derived here. However, the major experimental error affecting the present NMR determination of fatty acid composition is accuracy of integration. Controls of the accuracy of integration can be achieved by checking several other values. For example, the total integration value of all protons in the fatty acid chain (derived from the average fatty acid profile as reported in various literature; excluding the glyceridic or other ester protons) should be approximately 31.5 for soybean oil when assigning an integration value of 1 to each proton. This value varies slightly depending on the fatty acid profile of the vegetable oil. Thus, cottonseed and sunflower oils also have

values of approximately 31.5, while palm oil and canola (low-erucic rapeseed) oil display a value of approximately 32 and linseed oil, which is rich in linolenic acid (and thus has less protons) has a value of approximately 30.2. However, for samples of unknown origin and/or unknown relative fatty acid profile, this approach using approximate total integration values is more difficult or not feasible at all. Another possible control of accuracy of integration is Eq. 13.

In the samples containing triacylglycerols (Tab. 2), both vegetable oils and prepared mixtures, the amounts of C18:2 determined by ^{1}H-NMR are consistently lower than by GC or the known composition. This is also the case for the methyl soyate sample but not for the prepared mixtures of methyl esters (Tab. 1).

The results in Tabs. 1 and 2 utilize the peaks of the allylic protons (Eq. 10) rather than those of the olefinic protons as in the former case the results agree significantly better with prepared compositions and GC results. This effect was also observed in a previous study [17]. Another advantage of not using the olefinic protons is the proximity of the signals of the olefinic protons to the signal of the *sn*-2 proton in the glycerol backbone of samples containing triacylglycerols, which may overlap in some cases. However, the results for the individual saturated compounds often deviate from the known or gas chromatographically determined compositions although the total amounts of saturates are reflected accurately by ^{1}H-NMR. The reason for the greater deviation of the determination of the individual saturates by ^{1}H-NMR is the sensitivity of the integration. While the integration value of the methylene protons is the greatest of all integration values, the difference in integration values (Eqs. 22 and 23) is less than 5% of this integration value. Thus small changes in the total integration value are disproportionately reflected in the amounts of the individual saturated compounds. The deviation was verified for samples consisting of prepared mixtures of methyl palmitate and methyl stearate. Using the equation

$$A_{C16:0} = (28 - I_{CH_2}) \times 25 \quad (27)$$

Giving the percentage of C16:0 (28 is the maximum integration value of the methylene protons of methyl stearate resonating at 1.2–1.4 ppm), the following amounts of C16:0 were calculated for some mixtures consisting only of methyl palmitate and methyl stearate: 33.83% (actual content: 29.84%), 57.15% (actual content: 50.10%), 70.58% (actual content: 69.80%). Thus, even for less complex samples consisting only of saturated fatty esters, there is significant deviation in ^{1}H-NMR based quantitation.

3.4 Other applications

The present method can potentially be used for other applications. Alternative parameters to the iodine value introduced recently [31], with an equation for determining the iodine value from the fatty acid profile may serve as an example. These alternative parameters are termed allylic position equivalents (APE) and *bis*-allylic position equivalents (BAPE). They are defined by

$$APE = 2 \times (A_{C18:1} + A_{C18:2} + A_{C18:3}) \quad (28)$$

and

$$BAPE = A_{C18:2} + 2A_{C18:3} \quad (29)$$

These parameters can thus be determined directly from the fatty acid composition as discussed above. However, the APE and BAPE can be determined directly from the integration values of the corresponding peaks as follows.

$$APE = 200 \times (I_{exper,allylic}/4) \quad (30)$$

Since the maximum integration of the allylic positions is 4 and the definition of the APE is that one percent "concentration" of an allylic position gives APE = 1 with each allylic or *bis*-allylic carbon carrying two protons. For Eq. 30 to hold, it is assumed that each unsaturated fatty acid has only two allylic positions, which is the case for oleic, linoleic and linolenic acids. For the BAPE then:

$$BAPE = 200 \times (I_{exper,bisallylic}/4) \quad (31)$$

As 4 is the maximum integration value for the *bis*-allylic protons derived from the 4 protons in the two *bis*-allylic positions of linolenic acid. The factor 200 in Eqs. 30 and 31 results from the observation that the maximum value for these parameters is 200.

Thus, for the methyl soyate investigated here, the APE and BAPE parameters are APE = 166.9 and BAPE = 69.35. Generally, higher BAPE values may indicate a greater tendency of a sample to oxidize.

In conclusion, the ^{1}H-NMR spectroscopic method presented here for determination of fatty acid composition (unsaturated fatty acids and total saturated fatty acids) yields results that generally are in good agreement with other methods such as GC. The method can be applied to triacylglycerols (vegetable oils) and methyl esters, obviating the need for derivatives when their preparation may be undesirable or inconvenient. The method may also be useful for samples that are not amenable to GC analysis. Eqs. 7, 8 and 10 quantitate C18:1, C18:2 and C18:3, respectively. The total amounts of unsaturated fatty acids are given by Eq. 11 or Eq. 12 while the total amounts of saturated fatty acids are given by Eq. 13.

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